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Highly Diverse Morbillivirus-Related Paramyxoviruses in Wild Fauna of the Southwestern Indian Ocean Islands: Evidence of Exchange between Introduced and Endemic Small Mammals

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ABSTRACT

The *Paramyxoviridae* form an increasingly diverse viral family, infecting a wide variety of different hosts. In recent years, they have been linked to disease emergence in many different animal populations and in humans. Bats and rodents have been identified as major animal populations capable of harboring paramyxoviruses, and host shifting between these animals is likely to be an important driving factor in the underlying evolutionary processes that eventually lead to disease emergence. Here, we have studied paramyxovirus circulation within populations of endemic and introduced wild small mammals of the southwestern Indian Ocean region and belonging to four taxonomic orders: Rodentia, Afrosoricida, Soricomorpha, and Chiroptera. We report elevated infection levels as well as widespread paramyxovirus dispersal and frequent host exchange of a newly emerging genus of the *Paramyxoviridae*, currently referred to as the unclassified morbillivirus-related viruses (UMRVs). In contrast to other genera of the *Paramyxoviridae*, where bats have been shown to be a key host species, we show that rodents (and, in particular, *Rattus rattus*) are significant spreaders of UMRVs. We predict that the ecological particularities of the southwestern Indian Ocean, where small mammal species often live in densely packed, multispecies communities, in combination with the increasing invasion of *R. rattus* and perturbations of endemic animal communities by active anthropological development, will have a major influence on the dynamics of UMRV infection.

IMPORTANCE

Identification of the infectious agents that circulate within wild animal reservoirs is essential for several reasons: (i) infectious disease outbreaks often originate from wild fauna; (ii) anthropological expansion increases the risk of contact between human and animal populations and, as a result, the risk of disease emergence; (iii) evaluation of pathogen reservoirs helps in elaborating preventive measures to limit the risk of disease emergence. Many paramyxoviruses for which bats and rodents serve as major reservoirs have demonstrated their potential to cause disease in humans and animals. In the context of the biodiversity hot spot of southwestern Indian Ocean islands and their rich endemic fauna, we show that highly diverse UMRVs exchange between various endemic animal species, and their dissemination likely is facilitated by the introduced *Rattus rattus*. Hence, many members of the *Paramyxoviridae* appear well adapted for the study of the viral phylodynamics that may be associated with disease emergence.

Wild rodents and bats together comprise more than 60% of all known mammalian species and have been found to carry many zoonotic viruses (1). Recently, these groups of mammals have been shown to host a broad spectrum of novel paramyxoviruses (2–8). These data have broadened our knowledge of the host spectrum of *Paramyxoviridae*, a diverse viral family which is currently divided into two main subfamilies: the *Pneumovirinae* and the *Paramyxovirinae*. Members of the *Paramyxoviridae* include the causative agents of the human diseases mumps, measles and other respiratory tract infections due to *Parainfluenza* viruses and metapneumoviruses, as well as a large number of viruses associated with disease in animals, such as Newcastle disease virus, canine distemper virus, rinderpest virus, and others. Additionally, paramyxoviruses have been linked to a number of recent emerging or reemerging disease epidemics with high mortality rates (9). Of these, henipaviruses (*Paramyxovirinae*) have emerged as human pathogens from fruit bat populations since 1994 in Bangladesh, Malaysia, and Australia (10, 11). Additionally, a novel rubulavirus-related virus, similar to those known to be hosted by fruit bats, has been described with the capacity to cause illness in humans (12), showing how as-yet unknown *Paramyxoviridae* in wild animal reservoirs may pose an important health risk.

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A virus's reservoir consists of often-complex host communities that participate in its propagation and maintenance (13). Dynamics of individual host populations contribute to viral prevalence (14, 15), circulation, and dispersal (16); however, virus adaptation to its host can limit the potential for transmission between species (17, 18). The dynamics of viruses associated with their phylogenetics have been shown to be driven by a large number of complex and interacting factors (19–21), including the genetic diversity, ecology, and population dynamics of their host communities.

The southwestern Indian Ocean (SWIO) region is recognized as part of a biodiversity hot spot (22). In Madagascar, endemic mammalian taxa (including all native terrestrial and a significant percentage of volant species) have colonized the island independently since its separation from the Gondwana supercontinent more than 150 million years ago (23). In contrast, the smaller and much younger oceanic islands (mainly of volcanic origin) located around Madagascar (Mascarene, Comoros, and Seychelles archipelagos) host a limited number of mammalian species, and all of the terrestrial forms have been introduced (24, 25). The islands of the SWIO have been subjected to rapid anthropological expansion resulting in the dispersal and invasion of nonendemic rats and mice of the family *Muridae* and shrews of the family *Soricidae* to these isolated island ecosystems (26, 27). On virtually all of the SWIO islands, these introduced small-mammal populations occur in often dense populations, and on Madagascar, they compete with and displace native animal communities (28), potentially promoting the exchange of introduced infectious agents. These aspects make the SWIO an ideal location for the study of pathogen exchange (29).

In a previous study (3), we reported paramyxovirus infection in SWIO bats. Here, we have expanded our investigation into the diversity of this viral family within bats and other small mammals of Madagascar and other SWIO islands. We find that novel and unclassified morbillivirus-related paramyxoviruses (UMRVs) are commonplace within regional small mammals. Important differences between the morbillivirus-related and henipavirus-related virus models are observed, and detailed phylogenetic analyses suggest a major role for introduced *R. rattus* in diffusing UMRVs between different mammalian orders and different islands. These results highlight that UMRVs are a good model for understanding viral dynamics within wild fauna, especially between rodents and bats, two major reservoirs of zoonotic pathogens.

MATERIALS AND METHODS

Field work and sample collection. A total of 597 small, nonflying mammals were captured on Madagascar at two sites over the course of 3 years (2010 to 2012), once on Mahé (Seychelles) (June 2011) and once on Mayotte (Comoros) (July 2012), and from different sites on La Réunion (September 2012 to March 2013). A total of 140 bats were collected from several locations in Madagascar in 2012. Details on animal capture techniques are provided in the Text S1 in the supplemental material, and those on sampling locations are provided in Table S3.

Ethics statement. All animals were manipulated in accordance with guidelines for the handling of wild mammals (30). This study benefitted from sampling efforts conducted in the context of an ongoing international long-term project to catalogue the terrestrial vertebrate fauna of Madagascar based on voucher specimens (31). All protocols followed the terms of research permits (see Acknowledgments) issued by national authorities: Ministère des Forêts et de l'Environnement, Madagascar National Parks, Département de Biologie Animale (Madagascar); Direction de l'Environnement, de l'Aménagement et du Logement (France); and the Seychelles Bureau of Standards (Seychelles).

Laboratory work. (i) Nucleic acid preparation. Samples of approximately 1 to 2 mm^3 of kidney, spleen, and lung tissues were dissected on sterile ice from each individual animal. These tissue samples were then combined in 750 μl of Dulbecco's modified medium (Gibco, USA) containing 2- by 3-mm tungsten beads and homogenized for 2 min at 25 Hz in a TissueLyser (Qiagen). Homogenized organ samples were then clarified by centrifugation at 10,000 × *g* for 5 min. Two hundred μl of the clarified medium was biologically inactivated in 200 μl of AVL buffer (Qiagen), and total nucleic acids were extracted using the EZ1 virus V2 minikit in an EZ1 biorobot (Qiagen). Total nucleic acids were reverse transcribed in the presence of random hexameric primers using the GoScript reverse transcription kit (Promega) according to the manufacturer's instructions.

(ii) PCR screening. cDNA from each sample was screened using a seminested PCR system that targets a partial sequence (~490 bp) of the L polymerase gene of respiroviruses, morbilliviruses, and henipaviruses (RMH) as described by Tong et al. (32) and previously exploited in similar studies (2–6). Negative controls were routinely incorporated, and PCRs were repeated independently to ensure no cross-contamination when using nested PCR protocols.

(iii) Statistical analyses. Differences in detected prevalence (defined as the proportion of animals tested from which the sequence of the RMH PCR product obtained had significant homology to known *Paramyxovirus* sequences in BLAST) were statistically analyzed using a two-tailed Fisher's exact test. $P < 0.05$ was considered statistically significant.

(iv) Sequencing. PCR cDNA products of the approximate anticipated size (450 to 500 bp) were purified using the Qiagen PCR purification kit and cloned into the pGEM-T vector system (Promega). Cloned PCR products were Sanger sequenced (Genoscreen) using M13 standard sequencing primers. The sequence quality of individual reads was assessed, and all sequences were processed using the Geneious Pro software package (33). DNA sequences obtained from at least three independent bacterial clones were aligned to correct for the majority of sequencing or PCR-introduced errors. Coinfection was defined when sequences from the same animal showed greater than 5% genetic dissimilarity; in these cases, at least three clones of each different sequence were obtained. Primer sequences were trimmed from the finalized sequences.

(v) OTU-based family-level phylogeny. In order to classify the detected paramyxoviruses, viral family-level phylogenetic analyses were performed. The search parameters "Taxonomic classification: *Paramyxoviridae*" and "Any Field: polymerase" were used in GenBank (15 December 2013) to generate the final comparative sequence data set. In total, 1,816 sequences were assessed for their compatibility via pairwise alignment against a reference sequence from the *Henipavirus* genus (JN255806). The Geneious Translational alignment tool, using the default ClustalW cost matrix, was used to align all compatible sequences. Sequences were trimmed to remove any free end gaps or entirely removed from the analysis if the obtained alignment did not provide at least 420 bp of non-gap overlap, leaving 1,192 sequences for phylogenetic analysis (final length after trim, excluding gaps, 438 bp). Internal gaps were permitted. Operational taxonomic units (OTU) were defined using mothur (34) with a genetic distance cutoff of 10%, generating 196 consensus sequences that spanned all known, classified, and unclassified paramyxovirus genera. Representative sequences were selected using mothur for each defined OTU, which were then used in the final phylogeny (see Fig. 3).

(vi) Sequence polymorphism. DNAsp v.5 (available at http://www.ub.edu/dnasp/) was used to calculate the estimates of genetic diversity, Pi and Theta, from subsets of the final nucleotide alignments, separated by host species.

(vii) UMRV (and henipavirus) phylogeny. Individual sequences from OTUs falling within the UMRV clade were extracted from the original sequence alignment and used for further phylogenetic analysis (see

Fig. 4 and 5; also see Fig. S2 in the supplemental material). *Salem virus* (JQ697837) was included as an outgroup reference sequence.

(viii) Phylogenetic analysis. All presented phylogenies were constructed using the same methodologies; from aligned sequence data sets, jModelTest v2.1.2 (35) identified GTR+I+G as the most appropriate substitution model for all phylogenetic analyses in BEAST (36). Parameter estimates for different clock models were assessed in Tracer v1.5.0 (36), and a strict molecular clock was used for all further analyses. An additional three replicates of 100,000,000 iterations then were performed using these optimal parameters. Trees and parameter estimate files for all independent replicates then were combined using LogCombiner (BEAST package), assigning a 10% burn-in, which left only converged parameter estimates from each repeat. The obtained effective sample size values for each parameter were all superior to 200. The final phylogeny was generated using TreeAnnotator, and trees were manipulated using FigTree v1.4.

(ix) Cartographic data. Distributions of hosts were downloaded from the IUCN Global Mammal Assessment (http://www.iucnredlist.org/). Maps were processed in the Quantum GIS 2.0.1 (Dufour) software package.

(x) Ancestral-state reconstruction. Bayesian phylogenies from each of the preceding clade-level analyses were resampled, and the resulting 4,000 trees were imported into Mesquite (version 2.75; http://mesquiteproject.org/mesquite/mesquite.html). Geographical origins and mammalian host categories were attributed to each sequence as discrete state characters. The number of reconstructed trait changes was calculated using the unordered parsimony assumption and averaged for each ordinal category and over all trees, as described in reference 4.

Nucleotide sequence accession numbers. All sequences used for the present analyses have been deposited in GenBank under the reference numbers KF245939 to KF246061, KF408256 to KF408261, and KF928225 to KF928265.

RESULTS

Sampling across the SWIO islands yielded a wealth of animal samples. A description of the animal species subject to trapping activities is provided in Text S2 in the supplemental material. A molecular epidemiology survey for paramyxoviruses was carried out on 732 samples from four different animal orders, Chiroptera, Afrosoricida, Rodentia, and Soricomorpha, collected over a period of 3 years (2010 to 2012) in the SWIO region, specifically Madagascar, La Réunion, Mayotte (Comoros), and Mahé (Seychelles). All screening data are summarized in Fig. 1.

Madagascar. A total of 391 small nonflying mammals belonging to three orders (Rodentia, Afrosoricida, and Soricomorpha) and 22 different species were sampled at two sites on Madagascar. These included endemic Nesomyidae rodents (*Eliurus*, *Gymnuromys*, and *Nesomys*) and Tenrecidae tenrecs (*Microgale*, *Oryzorictes*, *Hemicentetes*, *Setifer*, and *Tenrec*), as well as introduced Muridae rodents (*Rattus*) and Soricidae shrews (*Suncus*). The species captured at each of the two sites varied, although the species diversity was comparable. The numbers of taxa captured at each site showed little temporal variation during the different field visits, suggesting homogenous sampling between years; however, a larger number of *R. rattus* specimens were collected in Lakato in 2012 than in the previous years.

Of the Malagasy nonflying mammals screened for paramyxoviruses, 25% (98/391) tested positive. Shrew tenrecs (Tenrecidae) belonging to the genus *Microgale*, for which the majority of sampled animals were represented by *M. cowani* ($n = 73$) and *M. dobsoni* ($n = 54$), showed the highest rate of paramyxovirus detection (40% positive; 69/172). Of these, four animals were coinfected with at least two different paramyxovirus strains (see Table S1 in the supplemental material). Paramyxoviruses were also detected in rodents, including *R. rattus* (30% positive; 16/54) and *Eliurus minor* (11% positive; 12/111). Variation in paramyxovirus infection levels was observed over the 3 years of collections on Madagascar, most markedly in populations of *M. cowani*, where differences between consecutive years were strongly significant ($P < 0.001$ for 2010 to 2011 and $P = 0.001$ for 2011 to 2012) and paramyxovirus infections peaked in 2011, with 85% of animals testing positive (Fig. 2).

Of the 140 Malagasy bats screened, a total of 41 belonging to eight bat species endemic to SWOI (*Chaerephon leucogaster*, *Miniopterus griveaudi*, *Mops leucostigma*, *Mormopterus jugularis*, *Myotis goudoti*, *Otomops madagascariensis*, *Triaenops menamena*, and *Vespertilionidae* spp.) and from four bat families (Hipposideridae, Miniopteridae, Molossidae, and Vespertilionidae) tested positive for paramyxoviruses.

Animals not endemic to other SWIO islands. Nonendemic small mammals collected outside Madagascar included *R. rattus*, *R. norvegicus*, and *Suncus murinus* on La Réunion and *R. rattus* on Mayotte and Mahé; paramyxoviruses were detected at each of these locations (Fig. 1), with *Rattus* spp. showing the highest proportion of positive animals (22%; 42/187).

Genetic and phylogenetic analyses. Phylogenetic analysis based on OTUs was sufficient to generate a well-supported, family-level phylogeny containing monophyletic groups for all main *Pneumovirinae* and *Paramyxovirinae* genera (Fig. 3). From this phylogeny, 10 clades were defined based on well-supported nodes (posterior probability [PP] > 0.8) that provided definitions at the taxonomic level of genus based on current taxonomic classifications. All of the 177 paramyxovirus sequences from the SWIO were members of a single genus-level group (UMRVs).

Absolute levels of genetic divergence were comparable between henipaviruses, morbilliviruses, and the UMRVs (see Fig. S1 in the supplemental material). Partial L-gene sequence divergence was calculated for those host species groups that were well represented in global sequence data and of interest for this study (see Table S2 in the supplemental material). Interestingly, less genetic divergence was observed between viral sequences originating from host species of different bird and mammalian orders (Aves, Afrosoricida, Rodentia, Soricomorpha, and Scandentia) than between viral sequences originating from within the Chiroptera, suggesting that bats can be infected by a broader range of paramyxoviruses than other hosts (Table 1). However, within the UMRV group, the genetic diversity was comparable between viral sequences from rodents and bats (Table 2).

Further phylogenetic analyses of all sequences that fell within the UMRV OTU-defined subgroups indicate that the detected paramyxoviruses comprise a certain level of host specificity at the level of host order (Fig. 4; also see Fig. S2 in the supplemental material). Two well-supported clades contain the majority of sequences originating from rodents, an additional two clades include most sequences originating from bats, and one final clade contains the greater part of sequences from tenrecs. Geographical clustering within the phylogeny was less apparent apart from viruses found in Afrosoricida, which are endemic to Madagascar (Fig. 5; also see Fig. S2). Cartographic analysis of the distributions of those species identified as hosts of the different UMRV groups (Fig. 4) suggested the global dispersal of many of these viral agents.

Members of the UMRVs included the Jeilam viruses (J, Beilong, and Tailam), viruses of rodent origin isolated in Australia

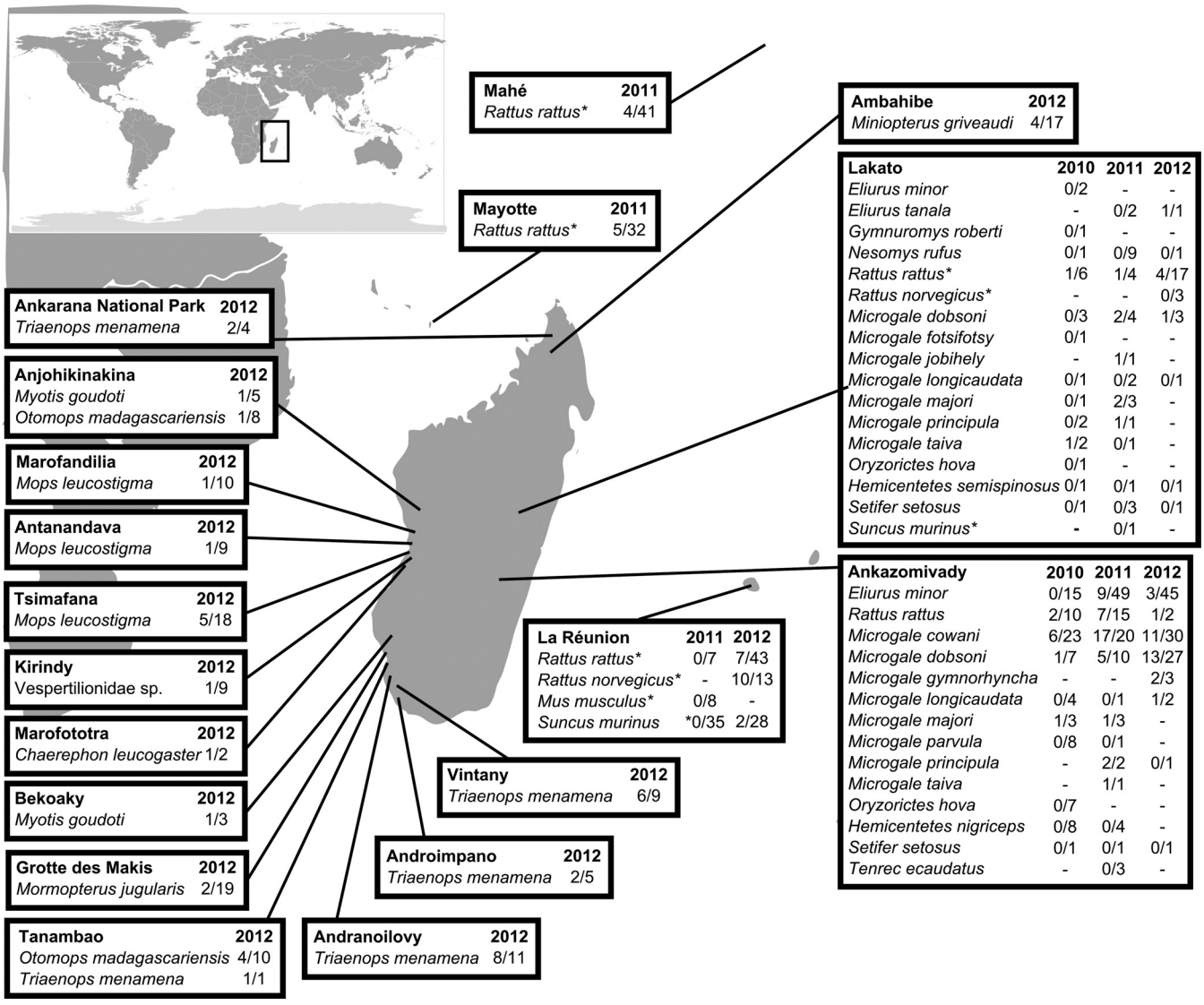

FIG 1 Paramyxovirus detection at different sampling sites in Madagascar and the SWIO. Numbers of positive animals, total numbers of sampled animals, and the year of capture are indicated for each sampling site. Species names preceded by an asterisk represent introduced taxa.

and China. Genetic and virological evidence has previously demonstrated that these viruses belong to the same taxonomic group (37–39). Interestingly, a strongly supported phylogenetic substructure separated Beilong and J viruses (see Fig. S2 in the supplemental material). The well-supported monophyletic group uniting these two viruses (PP = 1.0), designated rodent group 2 in Fig. S2 in the supplemental material, contained a further 71 paramyxovirus sequences, which were obtained from rodents and other small mammals from across the globe. Further well-supported substructures within rodent group 2 demonstrated differences in geographical distribution and host species association; for example, one subgroup contained only sequences obtained from the southern African rodent *Rhabdomys pumilio* (Muridae), whereas Beilong and Tailam viruses branched closely with *Rattus* species-derived sequences from the SWIO.

Ancestral-state reconstructions of UMRVs. Ancestral-state reconstructions were used to estimate the number of viral host switches and geographical exchanges based on the phylogeny presented in Fig. 2. These data are presented in donor/acceptor probability heatmap form in Fig. 4. Host-switching events were predicted to be most frequent between closely related species and between species belonging to the same order. *R. rattus* was predicted to participate in the largest number of host-switching events, being both the principle donor and principal acceptor species. *R. rattus* was also predicted to be involved in the majority of host exchanges that occurred between distantly related animal orders. Notably, elevated numbers of bidirectional viral exchanges between *R. rattus* and the Tenrecidae (*Microgale* spp.) were predicted to have occurred, and bats were also predicted to have received paramyxoviruses from *R. rattus*.

The geographical flux of paramyxoviruses was predicted to have occurred at least five times more frequently between Africa and the SWIO than any other continental regions. Exchange was also predicted to be frequent between SWIO islands, in particular between Madagascar, La Réunion, and the Comoros. It is important to note that our ancestral-state reconstructions could be in-

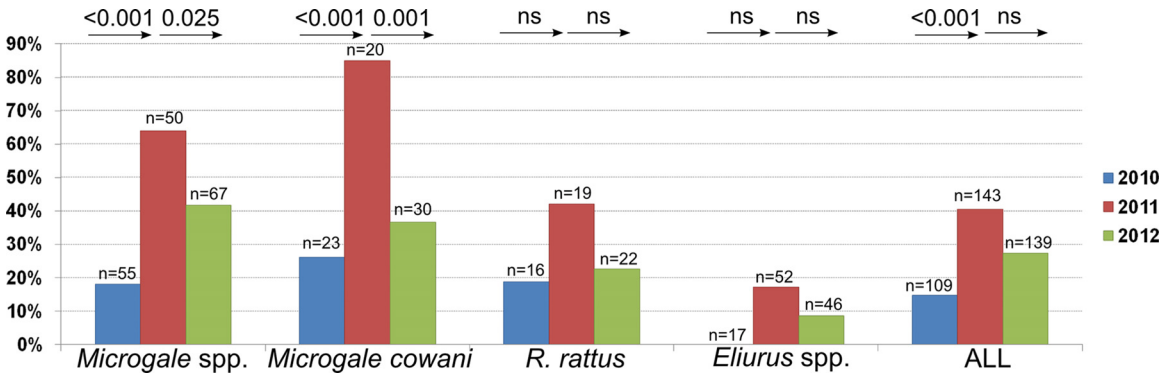

FIG 2 Temporal variation of detected paramyxovirus infection levels in animals from Madagascar. The number of paramyxovirus-positive animals is indicated in percentages. The total number of animals tested for each year is indicated above the bars. Colors are used to highlight the year of sampling, as described in the legend. *P* values displayed above each graph indicate the significance of differences between consecutive years (two-tailed Fisher's exact test; ns, not significant).

fluenced by a possible overrepresentation of paramyxovirus sequences from the SWIO; however, it should be noted that other large-scale screening efforts across the world have also contributed considerable data (2–6), originating from both rodents and bats and distributed across most known paramyxovirus genera (see Table S3 in the supplemental material). The limitations of similar analyses have been discussed in detail elsewhere for paramyxoviruses (4) and lyssaviruses (40).

For comparative purposes, similar analyses were performed on sequences from the henipavirus and unclassified henipavirus-related virus (UHRV) genus of the *Paramyxoviridae* (see Fig. S3 in the supplemental material). Here, host exchanges corresponding to human disease emergence via pigs and horses could be reliably predicted from the generated phylogeny, and geographical exchanges predicted an African origin for the henipaviruses, as well as large-scale circulation on the African continent, in agreement with other published literature on henipavirus biogeography (4, 8).

DISCUSSION

We have shown that many lineages of *Morbillivirus*-related paramyxovirus are in active circulation between islands in the SWIO. Bats and terrestrial small mammals play host to a number of highly divergent paramyxovirus strains belonging to this UMRV group.

Large numbers of paramyxovirus-positive animals were detected in this study. Endemic *Microgale* spp. and *Triaenops menamena* were identified as important paramyxovirus hosts, where 40% and 63% of animals, respectively, tested positive. In regional populations of introduced *Rattus* spp., this figure was

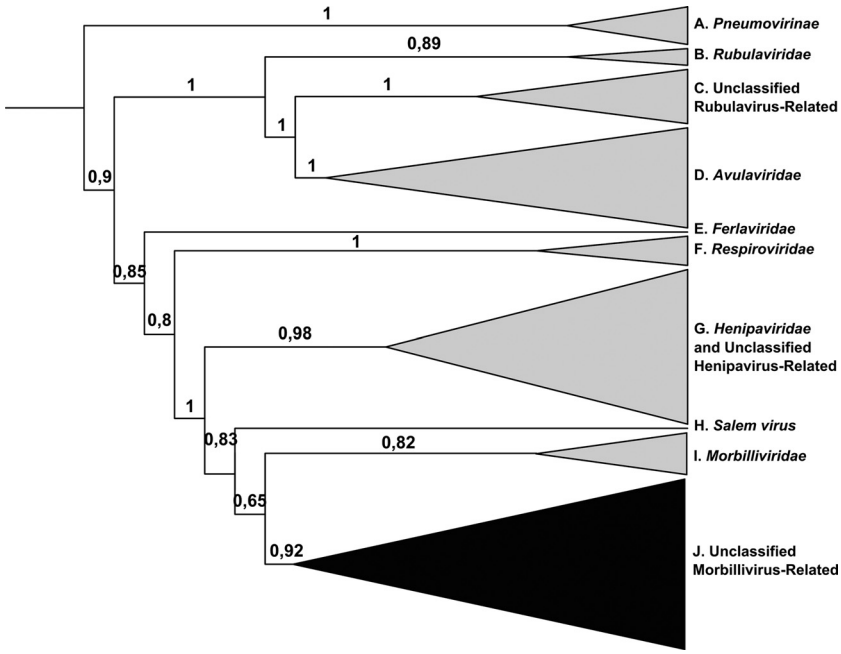

FIG 3 Paramyxovirus clades of the SWIO. (A) OTU-based family-level phylogeny of paramyxoviruses. The presented phylogeny is a proportional, ordered representation of data generated in BEAST. Relevant posterior probabilities (>0.5) are shown above each branch for clarity. Sequences are grouped into functional clades based on node support and height (see the text). Each functional group is assigned a letter (A to J). All sequences obtained within this study fell within group J, which is highlighted in black.

TABLE 1 Genetic diversity of the *Paramyxoviridae* by host order[a]

Parameter	Viral diversity in host order(s):			
	Aves	Chiroptera	Rodentia	Scandentia, Soricomorpha, and Afrosoricida
Pi (SD)	0.21123 (0.01385)	0.38181 (0.00730)	0.25410 (0.00947)	0.32354 (0.01149)
Theta	0.32854	0.35262	0.26267	0.33052

[a] Sequences from mammalian orders Scandentia and Soricomorpha are grouped with sequences from Afrosoricida due to the small number of viral sequences originating from animals of these orders. Pi is an estimation of nucleotide diversity, and Theta is equivalent to the Watterson estimator of the population mutation rate.

20%. A recent study (4) reported that *R. rattus* in Gabon and Thailand and *R. norvegicus* in Germany had low levels of infection (0/113, 1/49, and 0/131, respectively) when employing similar detection methods. However, the same study reported elevated numbers of paramyxoviruses in southern African *Rhabdomys pumilio* populations (17% of 512 animals tested). Additionally, another study conducted in Zambia has recently reported comparably high paramyxovirus infection levels in rodent (19%) and shrew (40%) populations (7). It is also of note that no henipavirus-related viruses were detected as part of this study despite the existence of serological evidence for the circulation of henipaviruses in Madagascar (41); however, members of this viral genus most often are associated with Pteropodidae fruit bats, which were absent from our samples.

The genetic diversity of partial L-gene sequences studied here is notably high, which is suggestive of the circulation of a large number of previously uncategorized viral strains (4). Additionally, multiple viral lineages could be identified within individual hosts, suggesting a level of compatibility between infecting viral strains. The overall observed genetic diversity of all paramyxoviruses per host was highest within the Chiroptera, suggesting that bats host a broader range of paramyxoviruses than terrestrial small mammals, in agreement with a recent hypothesis that bats are more permissive to viral infection than rodents (1). However, within the UMRV genus the level of viral diversity was similar between rodents and bats, showing that the relative importance of each host species likely is viral genus specific.

Different factors that may drive the observed levels of high genetic diversity of paramyxoviruses include the following: (i) the inherent genetic drift associated with many RNA viruses (42), which alone may be insufficient to explain high genetic variation between closely related viral lineages; (ii) coinfection within individual hosts, which is a "prerequisite for genetic exchange between different pathogen species or strains" (43), was observed in this and other studies of novel *Paramyxovirus* genera (2–4), and the possibility of recombination events between highly similar viruses should not be excluded, although their observation is rare for members of the *Paramyxoviridae*; and (iii) viral adaptation due to exchange within multihost systems (18).

The observed phylogenetic structuring of a divergent viral population of Jeilam-related viruses suggests that these closely related lineages have followed separate ancestral paths, generating a population structure similar to that of previously reported viral metapopulations (44, 45). Furthermore, analysis of the overall sequence diversity within well-defined *Paramyxovirus* genera suggests that the studied small fragment (~450 bp) is prone to genetic diversification compared to other loci of the paramyxovirus genome (46). Thus, in the absence of further virological or genetic data, the taxonomy of these viruses remains imprecise. In contrast, the observed genetic diversity, in association with considerable data originating from sources across the globe, has proven efficient for tracing the evolutionary paths of different viral lineages.

Using previously proposed terminology (13), the abundance of observed paramyxovirus infections suggests that animals of the SWIO islands constitute a putative paramyxovirus maintenance community. We predict, based on the reported virus-host interactions, that interspecies contacts are frequent within the natural ecosystems of Madagascar, in turn promoting what has elsewhere been referred to as a disease hot spot (22). For example, within 125 km of our two study sites, 12 *Microgale* species occur in syntopic and densely packed ecosystems (47). Thus, a considerable number of phylogenetically closely related host species, as well as animals of different mammalian orders, interact within dense and closed biotic systems, and host-specific adaptation appears to impose few barriers to viral host switching, resulting in frequent viral spillover and persistence within a multispecies community.

Viral persistence within a classical model source/sink community can be accompanied by genetic adaptation that establishes efficient virus-host interactions (17, 48); this may eventually create an evolutionary barrier that inhibits further host switching and results in the establishment of a new source population. This evolutionary process becomes complex for multihost ecosystems that exhibit frequent bidirectional host switching, such as that observed on Madagascar. Numerous factors will influence the efficiency of viral spreading within any studied community, including (i) viral and host abundance, (ii) host fitness, (iii) infection cost to the host, and (iv) the frequency and mode of transmission events (48). Infection dynamics within reservoir populations also directly influence viral epidemiology, with peaks of infection resulting in increased viral transmission (16). Here, the proportion of paramyxovirus-positive *Microgale cowani* increased 4-fold be-

TABLE 2 Genetic diversity within the UMRV genus per host group[a]

Parameter	Genetic diversity in:			
	Chiroptera	*Rattus* spp.	Rodentia	Afrosoricida (Tenrecidae)
Pi (SD)	0.28304 (0.00637)	0.24013 (0.01689)	0.28515 (0.00767)	0.22637 (0.00964)
Theta	0.27860	0.23846	0.25717	0.22740

[a] Pi is an estimation of nucleotide diversity, and Theta is equivalent to the Watterson estimator of the population mutation rate.

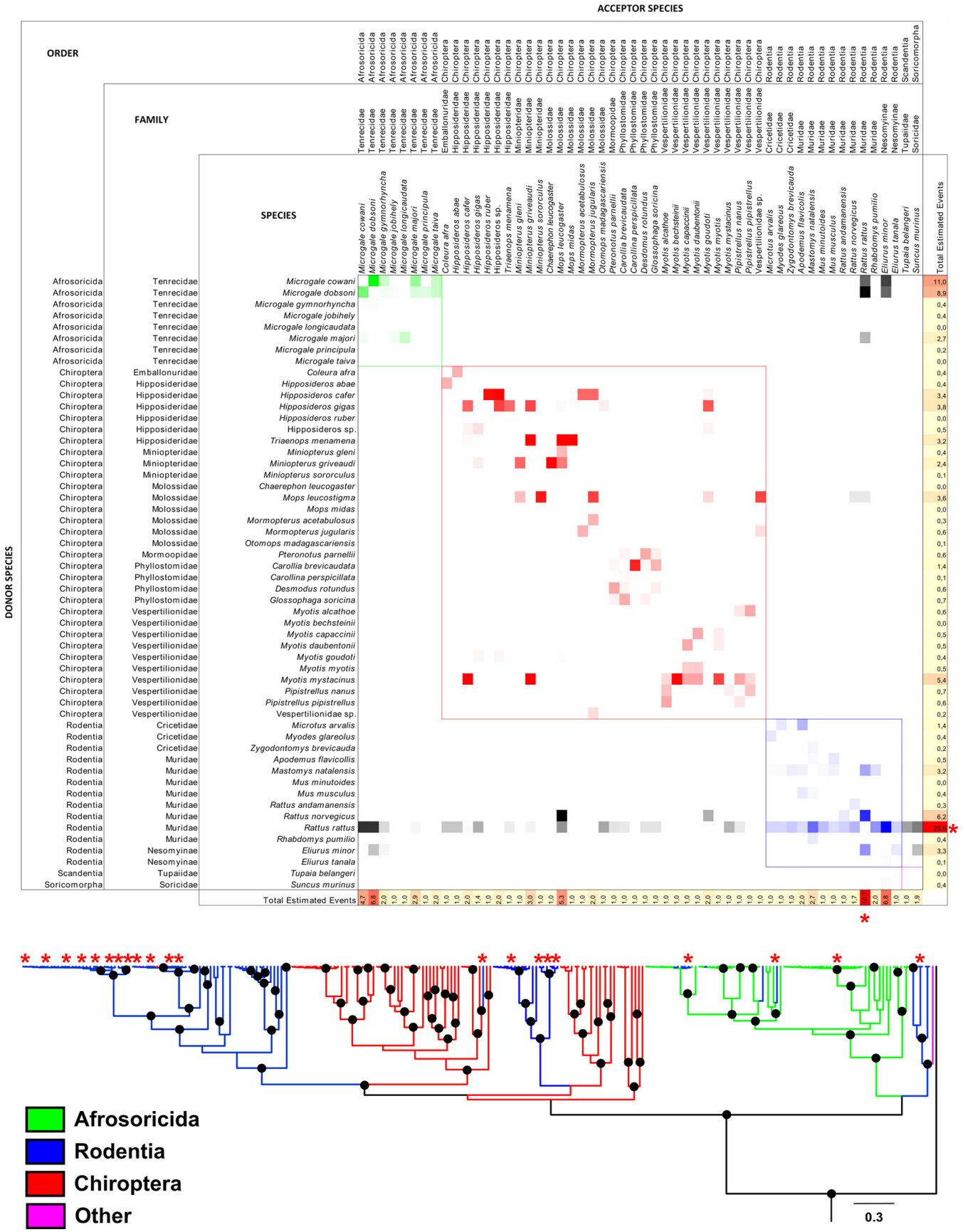

FIG 4 Ancestral-state reconstructions: host switching. The number of estimated host-switching events between species, based on parsimony analyses in Mesquite, are represented in tabular heatmap form. The presented phylogenetic tree is identical to that in Fig. 5 and Fig. S2 in the supplemental material and presents the most likely arrangement of the phylogenies used to estimate host-switching numbers. Dots represent nodes with posterior support greater than 0.9. Colors denote host-species order throughout. Red stars indicate positions of *R. rattus* in the phylogeny and the table.

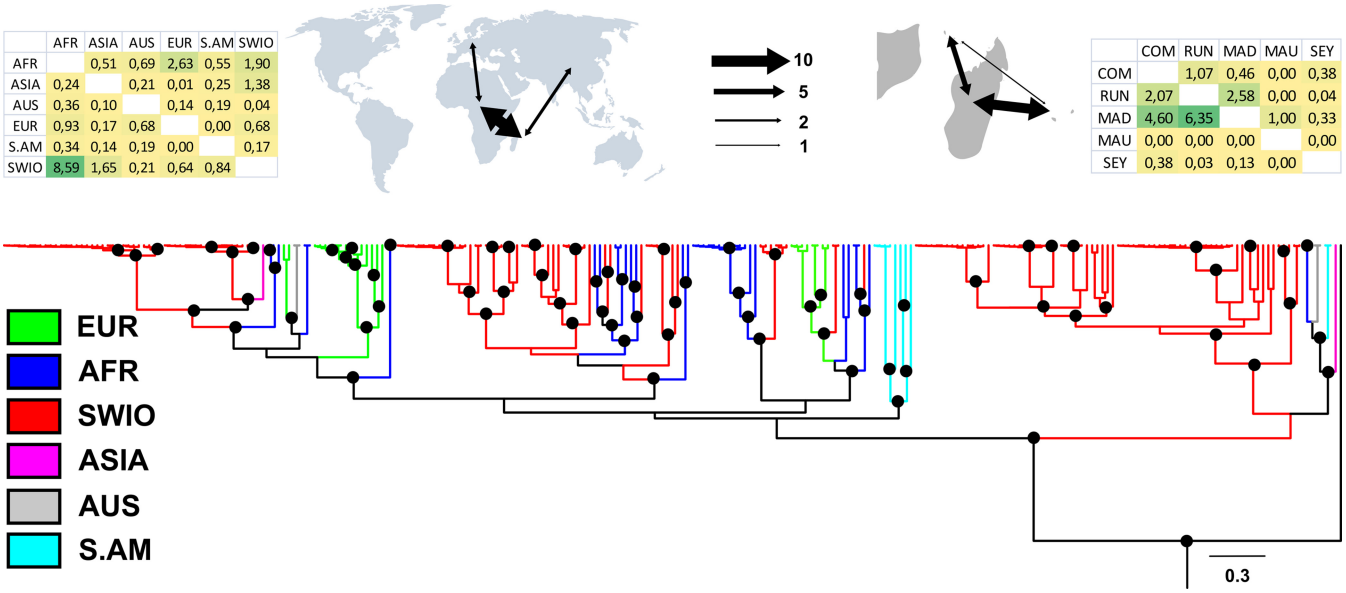

FIG 5 Ancestral-state reconstructions: geographical exchange. The number of estimated geographical switching events between sites based on parsimony analyses in Mesquite are represented in tabular form and are heatmap colored based on the estimated frequencies. The presented phylogenetic tree is identical to that in Fig. S2 in the supplemental material and Fig. 4 and presents the most likely arrangement of the phylogenies used to estimate geographical exchange numbers. Dots represent nodes with posterior support greater than 0.9. Colors depict continental origins.

tween 2010 and 2011, coinciding with the detection of closely related viral lineages in other host species. Spillover is a critical process for understanding zoonotic transmission but has rarely been documented in wild animal populations.

In addition to circulation within insular ecosystems, viral reservoirs can be established over large host communities, aided by their widespread geographical dispersal. From our data, the estimated levels of UMRV exchange between SWIO islands were high compared to global transmission levels, implying active host-associated dispersal between the African continent, Madagascar, and other SWIO islands.

Bats are theorized to play crucial roles in the processes of viral evolution that may lead to disease emergence (49) and are important hosts for numerous paramyxoviruses. Unlike henipaviruses, whose geographical distribution can be largely explained by the dispersal of Pteropodidae fruit bats (50), the observed geographical distribution of UMRVs is unlikely to be attributed uniquely to the dispersal of those bat species from which these viruses have been detected and most likely relies on interaction with other animal hosts. The data presented here indicate an important role for *R. rattus* in the transmission of UMRV paramyxoviruses and highlight these animals as potential intermediates in the dissemination of infectious agents that are endemic or otherwise isolated that facilitate disease emergence. The global dispersal of *Rattus* and their associated biota have been studied in many different contexts (25, 51) and have particularly important implications within the SWIO (52), where many mammal species are endemic, particularly on Madagascar, and at risk from both macro- and microorganism invasions, and where active commercial trade results in considerable and largely uncontrolled exchange (24, 53).

Within the insular ecosystems of the SWIO, paramyxovirus infection provides an excellent model for the study of both viral population dynamics and biogeography. Geographical exchange and host-switching events are common, likely occurring over short time scales due to some ecological factors that promote gene flow within an expansive viral maintenance community. In this context, *Rattus* play the dual role of a viral maintenance population and spreaders. Further virological investigation into the evolutionary implications of our observations may go a long way to developing an understanding of viral evolution within complex multihost communities.

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We have no conflicts of interest in relation to the submitted work to declare.

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